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TITLE: Persufflation of composite tissue transplants

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14. ABSTRACT The objective of this project was to test whether persufflation--perfusion with gaseous oxygen--can extend the time that a composite tissue transplant can be preserved in the cold. This work used the rat groin flap microsurgical model, in which the femoral vessels serve as the vascular pedicle. Our results showed that static cold storage in University of Wisconsin organ preservation solution for twenty-four hours was insufficient to maintain the viability of flaps seven days post-implant. Persufflation for twenty-four hours neither improved nor worsened flap viability. Shortening the storage time to fifteen hours also did not alter the effect of persufflation on flap viability. No clear differences in tissue histology were observed between explants of cold-stored versus persufflated flaps.					
15. SUBJECT TERMS Persufflation; oxygen perfusion; cold storage; rat; microsurgery					
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1. INTRODUCTION

Composite tissue transplants have shown remarkable results in treating large tissue deficits that result from major traumatic injury. The current standard is to preserve procured tissues in static cold solutions during transport to the operating theater, which limits the storage time to a maximum of 4-6 hours. This project sought to extend the preservation time by using persufflation—perfusion with gaseous oxygen—as the preservation technique in a rat model of composite tissue transplantation. The motivation for this approach originated from published studies that showed promising results in whole organ transplantation (particularly, for hypothermic kidney preservation).

This project compared the transplantation efficacy of persufflated versus statically stored rat groin flaps. It also optimized the persufflation procedure, particularly by altering the persufflation time. The targeted preservation time for this work was 24 hours, which would more than triple the current time limit; successful realization of this objective in the rat transplantation model would lay the groundwork for future large-animal and human studies.

2. KEYWORDS

Persufflation; oxygen perfusion; cold storage; rat; microsurgery

3. ACCOMPLISHMENTS

What were the major goals of the project?

The project had two major goals and three milestones, as detailed in the Statement of Work:

1) To compare the efficacy of static cold storage versus persufflation for preservation of composite, adipomusculocutaneous rat groin flaps.

Milestone: Development of procedures for removal, cold storage, persufflation, and transplantation of flap tissue.

Target date: Month 2

Status: Completed

Milestone: Comparison of persufflation versus static cold storage for groin flap transplantation.

Target date: Month 6

Status: Completed

2) To optimize the persufflation technique for 24-hour composite tissue preservation.

Milestone: Determination of optimal persufflation conditions.

Target date: Month 18

Status: Completed

What was accomplished under these goals?

In Year 1 of the award, we developed surgical procedures for the removal, cold storage,

persufflation, and transplantation of flap tissue, and began comparing the effectiveness of persufflation versus static cold storage for flap transplantation. We routinely removed, preserved, and transplanted flaps on the femoral arteries from one Lewis (inbred) rat to another. These microsurgical implants showed that unoptimized persufflation was able to partially preserve flaps for twenty-four hours in the cold (4°C). Nevertheless, implants of persufflated flaps did not yield results equivalent to control surgeries (i.e., immediate replantation). Immediate replants led to healthy integration of flaps (Fig. 1, *left*). Flaps that were stored in static University of Wisconsin solution for twenty-four hours led to poor survival seven days after

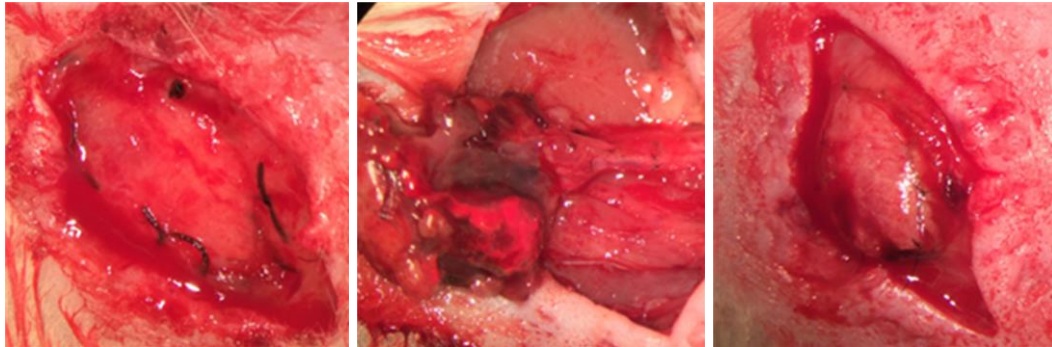


Figure 1. Intra-operative view of flaps seven days after implantation. (*Left*) Control replant (i.e., one that did not undergo cold storage). (*Center*) Flap that had been stored in static cold University of Wisconsin solution for 24 hrs. (*Right*) Flap that had been persufflated in the cold for 24 hrs.

implantation; in particular, thrombosis of the femoral pedicle was apparent (Fig. 1, *middle*). Flaps that were continuously persufflated (i.e., perfused with gaseous oxygen) during cold storage for twenty-four hours appeared to integrate well into surrounding tissue seven days after implantation, although regions of local hematoma were apparent (Fig. 1, *right*). These results suggested that while persufflation may be advantageous over static cold storage for flap preservation, it remained to be optimized.

Thus, in Year 2 of the award, we optimized the persufflation technique for 24-hour composite tissue preservation. Unfortunately, despite the initial promising results, we found that persufflation was not able to reproducibly extend the window of tissue preservation beyond that achievable by static cold storage. Even when the duration of storage was shortened to fifteen hours, both static cold storage and persufflation led to ~50% of transplants showing viability one week post-transplant. Figure 2 shows examples of viable (*left*) and non-viable (*right*) persufflated transplants.

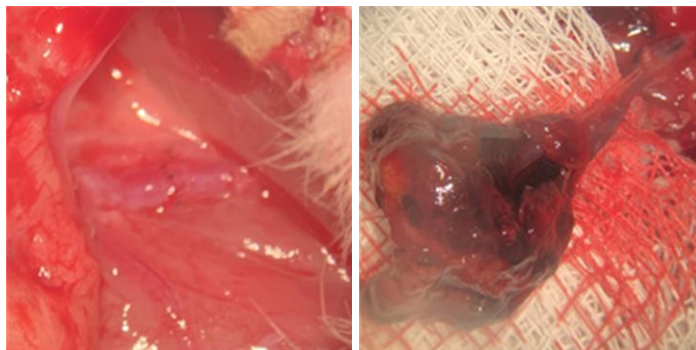


Figure 2. Intra-operative view of persufflated flaps seven days after implantation. Persufflation was for 15 hrs. (*Left*) Viable flap. (*Right*) Non-viable flap.

In the final year of the award, we performed a comprehensive histological analysis of persufflated and non-persufflated transplants, to determine if any clues for the lack of efficacy of persufflation could be obtained. These stains consisted of: standard H&E staining; periodic acid Schiff's, for polysaccharides; Mallory's phosphotungstic acid hematoxylin, for connective tissue; Movat's Pentachrome; Masson's trichrome stain; and Oil Red O, for lipid. No clear differences were observed between persufflated and non-persufflated transplants.

What opportunities for training and professional development has the project provided?

Although this work did not succeed in showing that persufflation could preserve flaps better than static cold storage did, it did provide extensive training and professional development for two personnel (Jing Xu and John Jiang). Both served as microsurgeons for this work. Mr. Xu is now a medical student at University of Massachusetts Medical School, and will most likely specialize in vascular surgery, in part as a direct result of his experience with this project. Mr. Jiang learned the techniques of microsurgical anastomosis in part from Mr. Xu and from the PI, and is currently an animal surgeon with another laboratory at Boston University.

How were the results disseminated to communities of interest?

To date, the results have not been disseminated publicly. Given that we did not find that persufflation improved tissue viability compared to static cold storage, it is unlikely that we will publish these findings.

What do you plan to do during the next reporting period to accomplish the goals?

Not applicable. The project has been completed.

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?

Nothing to report. Although our Science Officer suggested that we consider publishing the results, our view is that a null result is not publishable.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES / PROBLEMS

As mentioned in the previous progress reports, we ran a few months behind schedule,

mainly due to the initial slow pace of establishing a surgical routine. We also paused our work in early June 2019 pending approval for the no-cost extension (approval letter received from USAMRAA at the end of July 2019).

6. PRODUCTS, INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Publications, conference papers, and presentations

Nothing to report. Although our Science Officer suggested that we consider publishing the results, our view is that a null result is not publishable.

Website(s) or other Internet site(s)

Nothing to report.

Technologies or techniques

Nothing to report.

Inventions, patent applications, and/or licenses

Nothing to report.

Other products

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Joe Tien
Project Role: Principal Investigator
Researcher Identifier (e.g. ORCID ID): n/a
Nearest person month worked: 3.59
Contribution to Project: Dr. Tien directed the project and worked with John Jiang to perform the surgical procedures.

Name: John Jiang
Project Role: Surgeon (animal microsurgery)
Researcher Identifier (e.g. ORCID ID): n/a
Nearest person month worked: 2.58
Contribution to Project: Mr. Jiang performed the isolation, preservation, and transplantation of the tissues.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report.

What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

A final quad chart is attached.

9. APPENDICES—None.